Monoclonal expansions of TCR in a diabetic pancreas

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- *Case 1* is the pancreas from a diabetic donor dead 5 days after debut
- Infiltrating $CD8^+$ T cells were predominant compared with $CD4^+$. Analysis of TRBV repertoire showed a few dominant families (Somoza et al, 2004)
- At onset, genes related to inflamation were upregulated (Planas et al, 2009)




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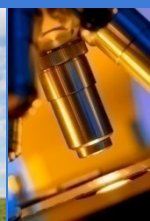

Aims

- Analysis of TRBV repertoire to identify monoclonal expansions
- Comparison of intrapancreatic infiltrating T cell (IPL) repertoire with autologous spleen T cells
- Study of Vβ11 (TRBV25) sequences from the pancreas, spleen and expanded T cell lines from IPLs




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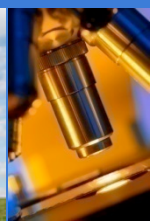
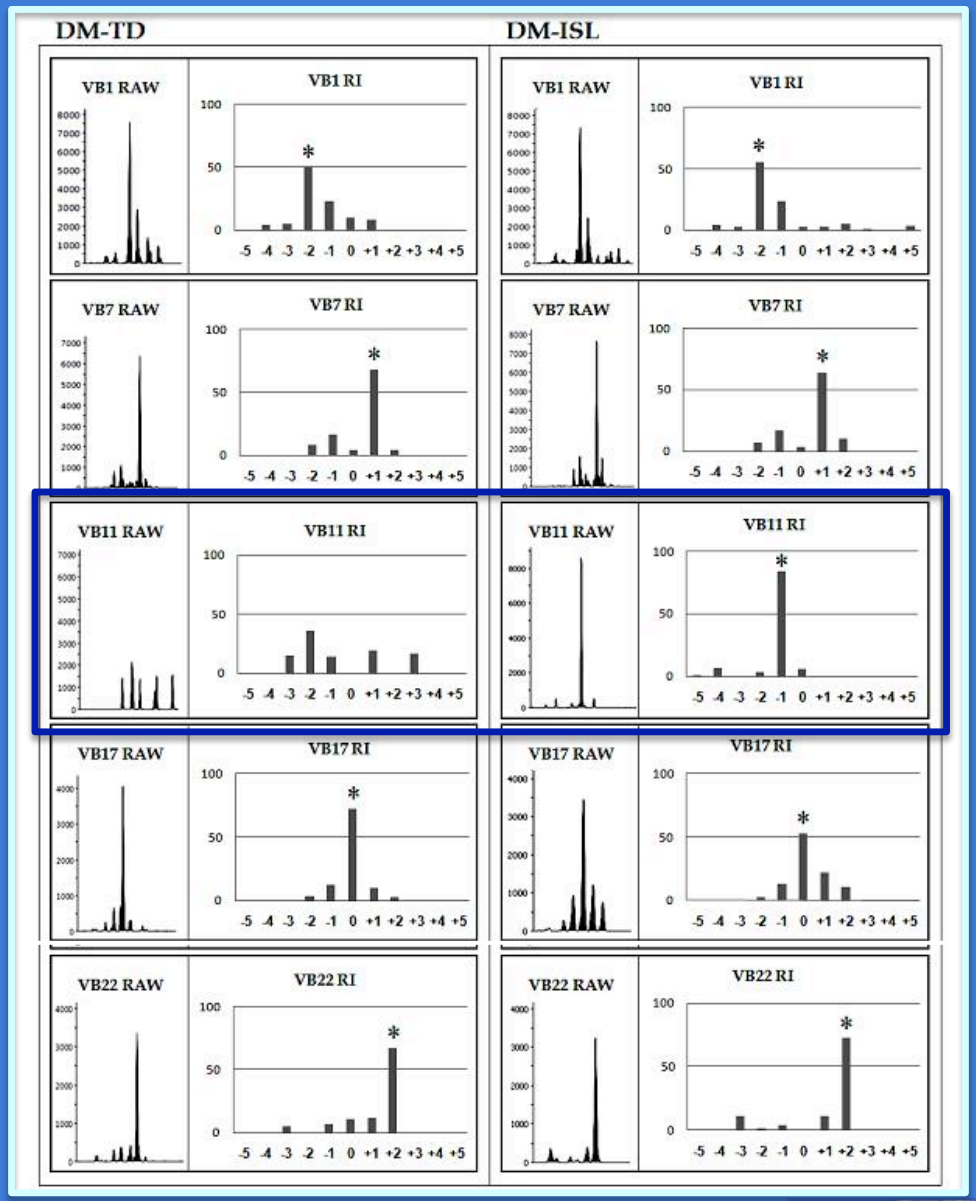


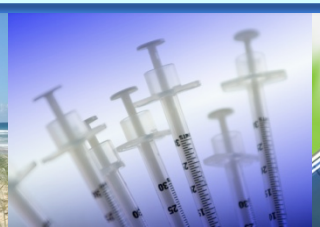


TRBV FAMILIES WITH MONOCLONAL EXPANSIONS (RI>50%) IN DM-TD AND DM-ISL SAMPLES

5 monoclonal expansions in Vβ families, with RI>50% (total area>10 and peaks representing more than 50% of the total area), corresponding to Vβ1, Vβ7, Vβ11, Vβ17 and Vβ22 families

Islet monoclonal expansions were also detected in whole tissue samples, except Vβ11 that was only expanded in islets

(Codina-Busqueta et al, J. Immunol 2011)



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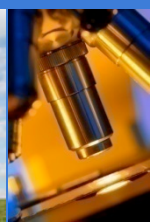
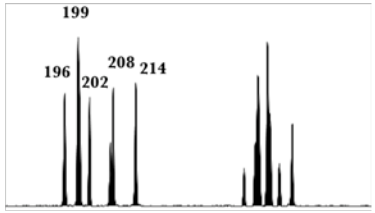


Vβ11 in pancreas and autologous spleen from *Case 1*

- **Samples from Case 1 (T1D patient)**

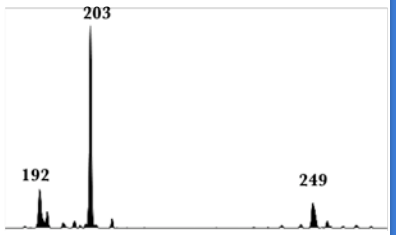
- **Pancreas (MIX D)**

DM-TD



Vβ11 Vβ5.1

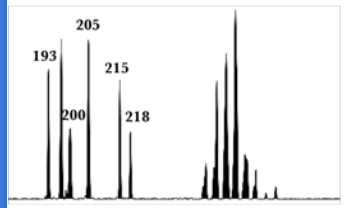
DM-ISL



Vβ11 Vβ5.1

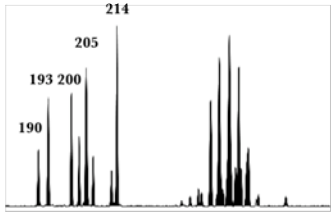
- **Spleen (MIX D)**

DM-S1



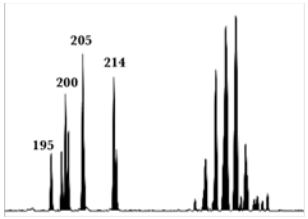
Vβ11 Vβ5.1

DM-S2



Vβ11 Vβ5.1

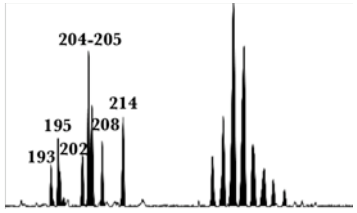
DM-S3



Vβ11 Vβ5.1

- **PBMCs**

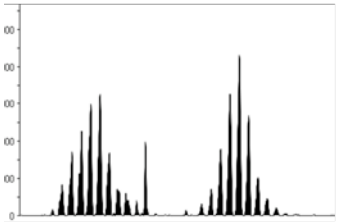
DM-PB



Vβ11 Vβ5.1

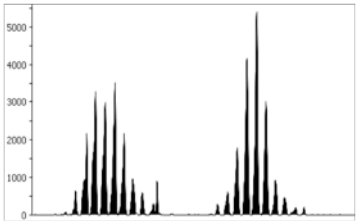
- **PBMC from healthy donors**

C-PBL1

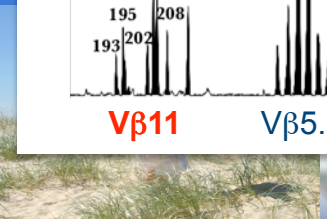


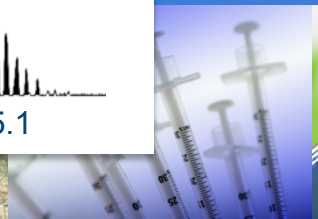
Vβ11 Vβ5.1

C-PBL2



Vβ11 Vβ5.1





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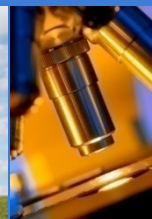
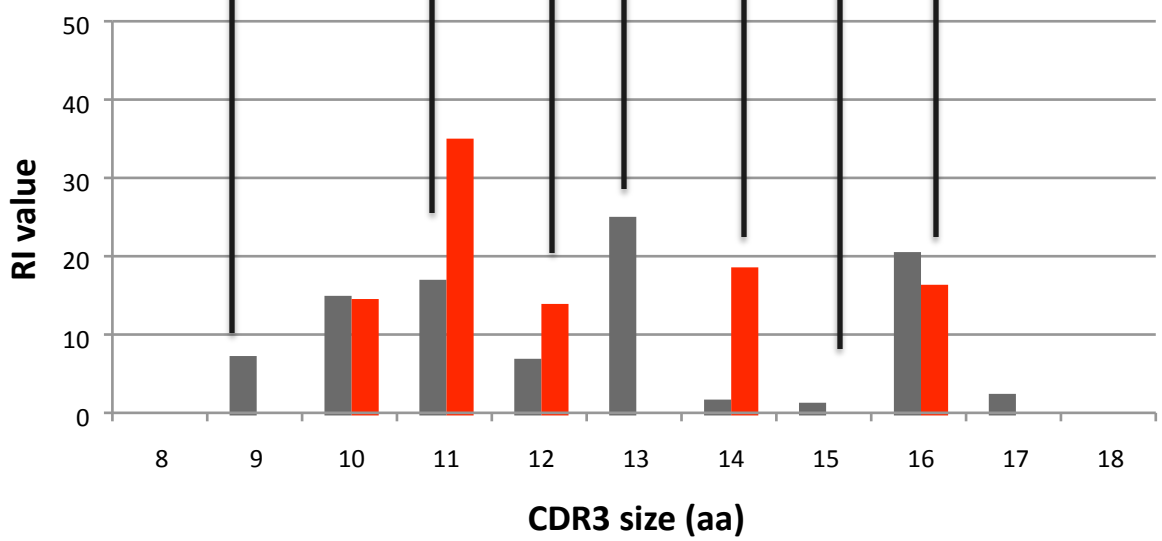


Vβ11 sequences from spleen and pancreas

CASSQNIEQFF(16%)
CASQVHYGYTF(21%)
CASSENNHGEQYF(4%)
CASSKGQGGGYTF(4%)
CASSLNLADTQYF(8%)
CASSVGRGYGYTF(4%)
CASSDTNTGELFF(20%)
CASSEGGQGARGYTF(8%)
CASSGRPRGNEKLFF(17%)
CASGGGTGGANEQFF(4%)
CASSDLRGDSPLHF(8%)
CASSDPGTQETQYF(4%)
(monoclonal expansion in islets 89%)
CASTPNKQGRGQPQHF(28%)
CASSELGGGGGTDTQYF(12%)
CASSDLDLQAGGIVGYTF(8%)
CASSEWGLSGEYQETQYF(17%)(20%)
Seq. found in both organs



Sequenced clones:
- spleen n=24
- total digest n=25

DM-S
DM-TD



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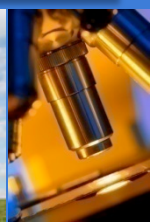
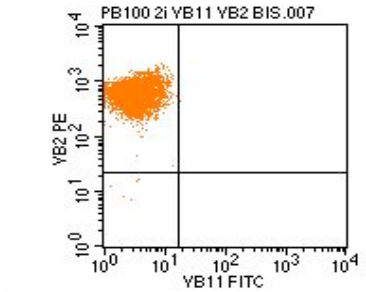
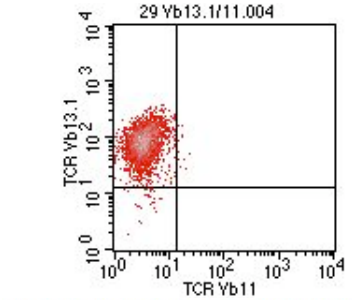


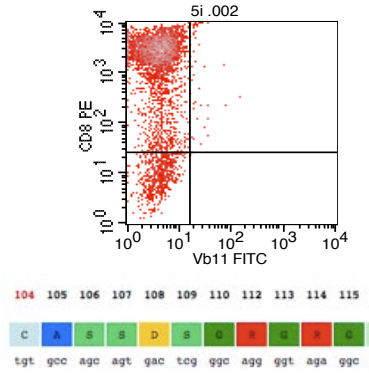
High frequency of Vβ11 in T cell lines expanded from infiltrating pancreatic lymphocytes

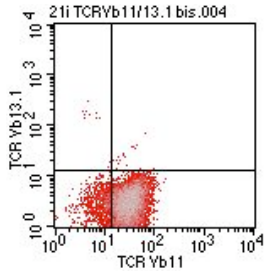
TRVB usage was diverse with predominance of Vβ11 acompanied by a second rearrangement. Vβ11 and Vβ13.1 pairs were the most frequent

FACS analysis showed that some Vβ11 T cell lines did not show Vβ11 protein expression, such as .2i ($CD4^+$), .29 ($CD4^+$) or .5i ($CD8^+$). Only a few showed surface protein expression (.21i).









Sequence not done

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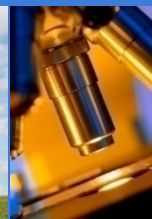
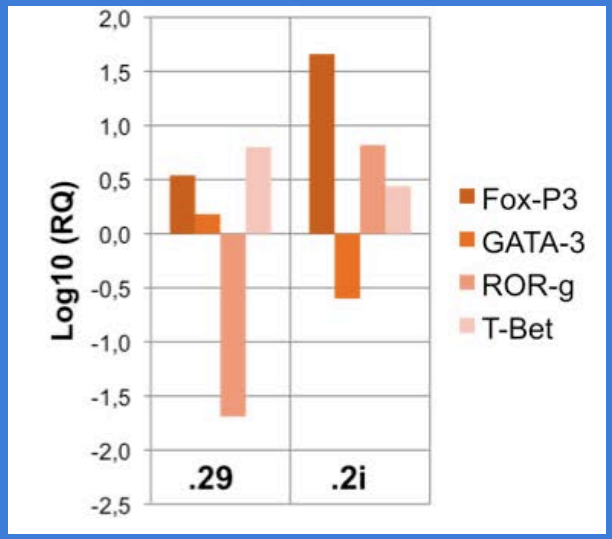
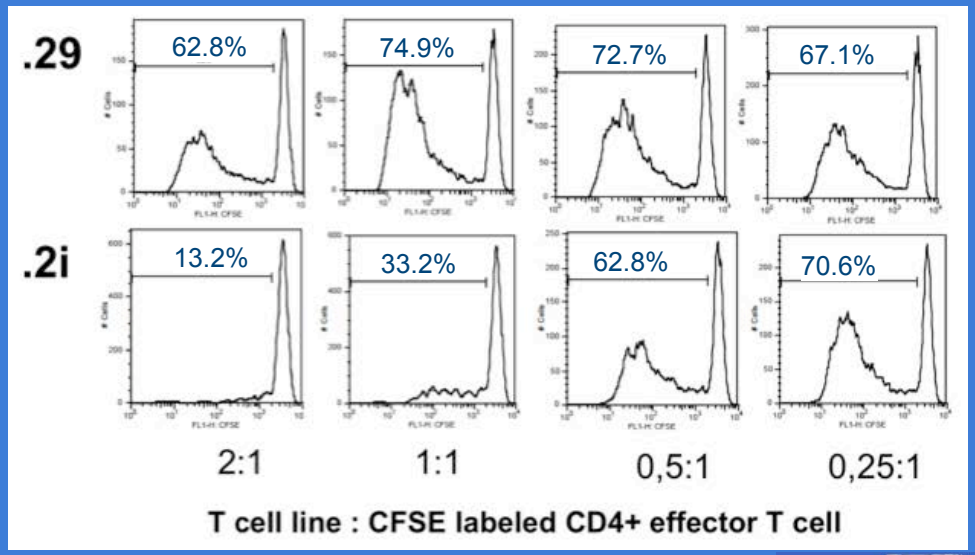


Functional characterization of Vβ11-expressing T cell lines .2i and .29



Transcription factor expression analysis by qPCR, using PBMC as reference value:

.29 expressed both T-bet and GATA-3 and .2i expressed FoxP3 and RORγ



.2i T cell line suppressed proliferation of CFSE-labeled $CD4^+$ effector T cells in the presence of anti-CD3/CD28 beads, in concordance with FoxP3 expression

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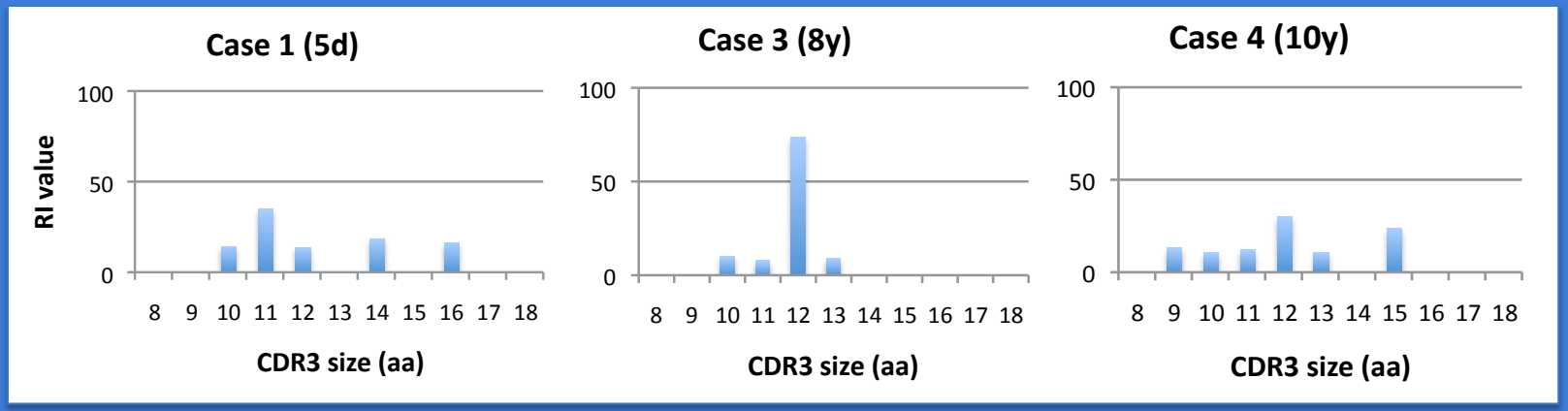
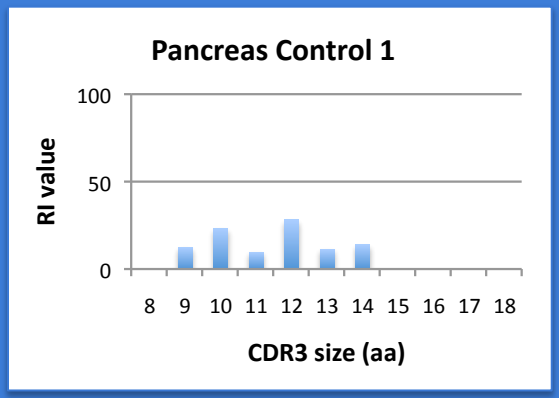
Vβ11 was also restricted in other diabetic pancreas and present in healthy pancreas





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CONCLUSIONS

- Five monoclonal expansions were defined in the intra-islet infiltrate from which the Vβ11 expansion (CASSDPGTQETQYF) was exclusive of islets.
- CDR3 sizes of Vβ11 did not follow a normal distribution in pancreas, spleen and PBMC samples from the diabetic patient (*Case 1*).
- Sequence diversity of Vβ11 in the pancreas was lower than in the spleen. The intraislet monoclonal expansion of Vβ11 was not found in spleen.
- Cell lines from diabetic infiltrate showed a preference for Vβ11 acompanied by a second rearrangement. Vβ11 was not expressed at the surface of most Vβ11$^+$ T cell lines.
- T cell lines coexpressing Vβ11 (CASTPNKQGRGQPQHF, sequence from islets) and Vβ13.1 showed a Th2-like phenotype and were Foxp3 negative.
- These putative non-functional rearranged Vβ11 transcripts could not be associated with any regulatory or effector function.
- Vβ11 family was also expressed in other diabetic pancreas but also in healthy donors.




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